Protocols Used at NCI & Related Information

Processing of Microdissected Tissue for Molecular Analysis Proteomics-based Studies

The methodology and buffers utilized for processing microdissected samples for protein-based studies is **variable** depending on the downstream molecular analysis method. As an example from our lab, for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) studies, the recovered cells are placed initially in:

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100 µl of IEF lysing solution containing 7M Urea 2M Thiourea 4% CHAPS 1% MEGA-10 1% Octyl-b-Glucopyranoside, 40 mM Tris 50 mM DTT 2 mM tri-butyl phosphine (TBP) 0.5% (v/v) Pharmalytes (pH ranges 3-10, 4-7, or 6-11)
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Place the buffer in an Eppendorf tube, place the LCM cap on the tube, invert the tube, and vortex vigorously for one minute until all cells are completely lysed.

For information pertaining to the sensitivity of proteomic-based approaches to analyze small numbers of microdissected cells, see <u>Proteomics Limitations</u>.